

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Yang, Yinong

Serial No.: 10/768,886

Art Unit: 1638

Filed: January 31, 2004

Examiner: Vinod Kumar

For: Mitogen-Activated Protein Kinase
And Methods for Use to Enhance Biotic
And Abiotic Stress Tolerance in Plants

Atty Docket No.: UAF-03-14

DECLARATION OF YINONG YANG, PH.D.

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

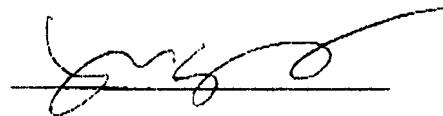
I, Yinong Yang certify the following:

1. I am the inventor of U.S. Patent Application No. 10/768,886.
2. I am an Associate Professor of the Department of Plant Pathology and the Huck Institutes of the Life Sciences at Pennsylvania State University.
3. I possess a doctorate of philosophy from the University of Florida and post-doctorate training from Waksman Institute of Rutgers University.
4. Since 1990 I have been working in the area of plant-pathogen interactions.
5. My current research focuses on the complex network of signal transduction involved in rice disease resistance and abiotic stress tolerance.
6. I have over 35 peer-reviewed publications in my research area.
7. I have reviewed the specification of U.S. Patent Application No. 10/768,886.
8. I have reviewed the Office Action dated May 15, 2007.
9. On or about May 2000, my laboratory isolated the gene fragment of OsMAPK5 (plasmid clone #2C12) (see attached Exhibit A, Lab Notebook I at pages 1-2).

10. On or about September 2000, my laboratory isolated the full length gene of OsMAPK5 (plasmid clone #M2) (see attached Exhibit A, Lab Notebook I at pages 3-5).
11. From approximately November 2000 to May 2001, RNA and protein analysis of OsMAPK5 indicating response to biotic and abiotic stresses were performed in my laboratory (see attached Exhibit A, Lab Notebook I at pages 6-7).
12. On or about November 2000, rice transformation was initiated for over-expression (H series) and suppression (F series) of OsMAPK5.
13. On or about May 2001, my laboratory began to obtain transgenic rice lines (see attached Exhibit B, Lab Notebook II at page 1).
14. During approximately, June 2001 to May 2002, two generations of transgenic rice lines were analyzed for disease resistance and abiotic stress tolerance (see attached Exhibit B, Lab Notebook II at pages 2-4).
15. Prior to studies in my laboratory, no one in the field was aware that rice MAPK5 gene, its protein and enzyme activity were induced by drought, salt and low temperature and capable of rendering abiotic stress tolerance.
16. The data filed with this declaration was generated from work performed in my laboratory at the University of Arkansas located in Fayetteville, Arkansas.

I certify that the foregoing statements made by me are true. I am aware that if any of the foregoing statements made by me is willfully false, I am subject to punishment.

Date: 9/14/2007



Yinong Yang Ph.D.

EXHIBIT A

Department Plant Pathology
Subject ^{Rice} Defense gene Screening and Ident
Name Lizhong Xiong (NTL)
Address R. APC 215

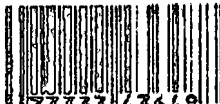
National Brand

99.10 - 2001.2

Computation Notebook

113/4" x 91/4", 4 x 4 Quad., 75 Sheets

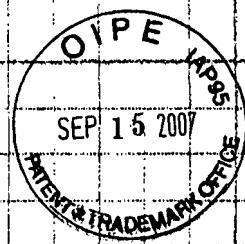
43-648



0 73333 43648 8

I

Office Products
Chicopee, MA 01022



May 3 Rice seeds (Drew) planting
53g → 60 plots pots

May 4 Blots probe
N5-1 N6-1
Minnow's chemical
Induced (including cmg)
seedlings
N5-2 N6-2
Minnow's blots
Suspension cell
BT110
JA-60

May 8-9 Culturing & Mini-prep / sequencing
↓ all Azo/20 ≥ 1.8 but ≤ 1.95

1	2A10	0.32243/41	11	2D10	0.29	21	2F11	0.34
2	2A12	0.34	12	2E2	0.31	22	2G1	0.32
3	2B1	0.28	13	2E3	0.30	23	2G5	0.37
4	2B7	0.36	14	2E4	0.5	24	2G6	0.38
5	2C1	0.30	15	2E7	0.41			
6	2C3	0.46	16	2E8	0.42			
7	2C4	0.38	17	2E11	0.37			
8	2C12	0.36	18	2F7	0.40			
9	2D2	0.46	19	2F8	0.29			
10	2D7	0.38	20	2E1	0.41			

Blast Result of JBC sequence

SX#	Inducible data			Possible genes based on homology (BLASTX)
	Blast	BTH	JA	
2A2		-	+	No homology
2A3	-	+	++	Putative Beta-ketoacyl-CoA synthase
2A4	-	+	++	Low homology (9E-5) with an unknown protein from Arabidopsis
2A8	+	+	++	No homology
2A10	-	+/	+	No homology
2A12	-	+/	++	gb AAF21081.1 AC013258_19 (AC013258) unknown protein [Arabidopsis thaliana]
2B1	-	+/	+	No homology
2B7	-	+/	+	1. hypothetical protein from Arabidopsis (5E-38) 2. cytokinin oxidase-like protein (Arabidopsis) (7E-24)
2B8	-	+/	+	hypothetical protein from Arabidopsis (5E-18)
2B9	-	-	++	No homology
2C1	+	-	-	No homology
2C3	++	+	-	RUBISCO activase
2C4	+	+	+	=2F8
2C12	++	+	+	MAP kinase (high homology one from maize)
2D2	++	++	-	(AC016661) Putative ankyrin (arabidopsis)
2D7	+	-	+	(S39045) Zinc-finger protein from wheat (WZF1) <i>Minerva</i>
2D10	+/	-	+	Hypothetical protein from Arabidopsis (4E-6), 24/32 (75%)
2E2		+/	+	(Z99707) MAP3K-like protein kinase from Arabidopsis
2E3	-	-	+/	Not sequenced
2E4	+++	-	++	No homology
2E7	R	+++	+++	Low homology: hypothetical protein from Arabidopsis
2E8	-	-	+	No homology
2E11	-	+	++	NAD-malate dehydrogenase
2F6	++	+/	+	Oryza sativa mRNA for osNAC6 protein (E-155)
2F7	+	+	++	No homology
2F8	+++	-	++	Beta-ketoacyl-CoA synthase
2F10	R	-	+	1. An unknown protein from Arabidopsis 2. Ca+2-binding EF hand protein from soybean 3. ABA induced protein from rice
2F11	+	+	++	= 2A12
2G1	++	+/	++	No homology
2G5	R	-	-	Chlorophyll A/B binding protein
2G6	++	-	++	(AF225703) RSH2: Arabidopsis Rel/SpoT homology

~~Adel~~

SX3A4

SX2B7

SX1 F1

- 2D8

For delete redundancy

Aug 5 MAP Kinase (2G2) Screening again.

Some (4 in 10) weak signal dots → Continue

AUG 15

Northern

ABJS 1

HW 1

blast 7th

Southern 2nd

L34 sp (specific probe obtained by PCR)

ABJS 2

HW 2

blast 9th

Southern 4

L68 sp (specific probe)

Phosphorimager's scan: nothing bands remained

→ washing problem

→ Blots problem (too much blots)

AUG 21

* Library screenings with L80 (partial or DNA insert 1 Kb or so)
* probe DNA was checked by gel

* Northern

ABJS 1

HW 1

blast 6th

Southern 2

L34 sp

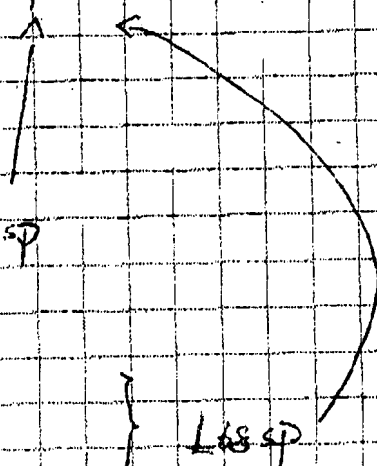
ABJS 2

HW 2

blast 10 (1st time use)

Southern 1 (1st time use)

L68 sp



9/30



10/2

Successfully excised all phagemid into plasmid.

XLOLR cell: new tube grown in LB.

Absolutely fresh cells be used.

$$M_{11-1} = [M_{11-2} = M_{11-3} = M_{11-5} = \underline{M_1}] = 1.4 \text{ kb}$$

$$M_2 = M_8 = 1.6 \text{ kb}$$

$$M_3 = 2.2 \text{ kb}$$

$$M_4 = 0.8 \text{ kb}$$

$$M_5 = M_6 = 1.3 \text{ kb}$$

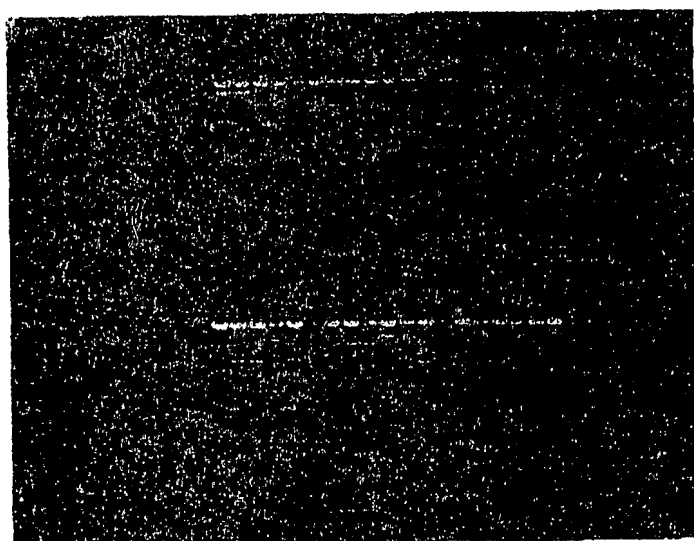
$$R_1 = 1.4 \text{ kb}$$

$$R_2 = 1.4 \text{ kb}$$

$$R_{41-1} \neq -2 \neq [-3 = -4 = -5]$$

may be true 1.5 kb

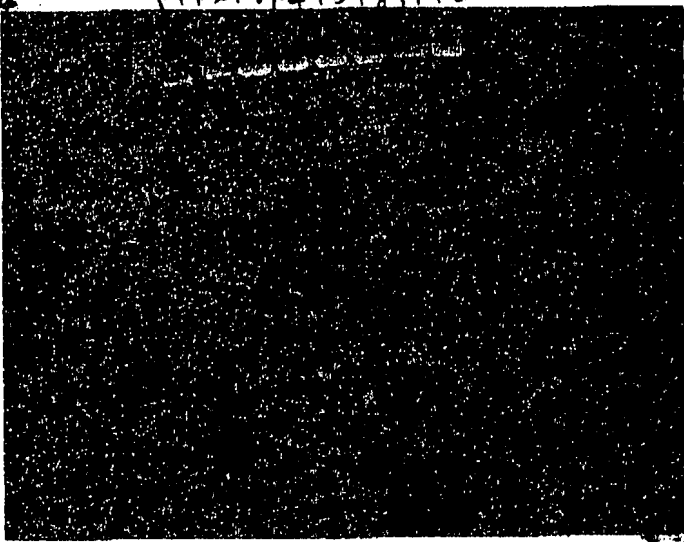
$$R_{51-1} \neq -2 \neq -3 = -4$$



Min. prep	Final conc.	
M ₁	0.16 mg/ml	1.85
M ₂	0.1	2.0
M ₃	0.36	1.62
M ₄	0.24	1.84
M ₅	0.25	1.7
M ₈	0.22	1.8
R ₁	0.11	2.2
R ₂	0.26	1.8

10/14

M₁ M₂ M₃ M₄ M₅ M₈ R₁ R₂



Send 6 samples for sequence (To little rock)

No. 6 M₂ = M₈ ? 2 G₁₂ (need further sequence or digestion)
 No. 1 M₃ — Wrong! → 18S RNA
 2 M₅ partial ? = M₂ or M₈
 3 M₈ = M₂
 4 R₁ fuller length ? = 280

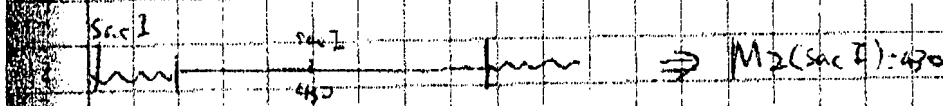
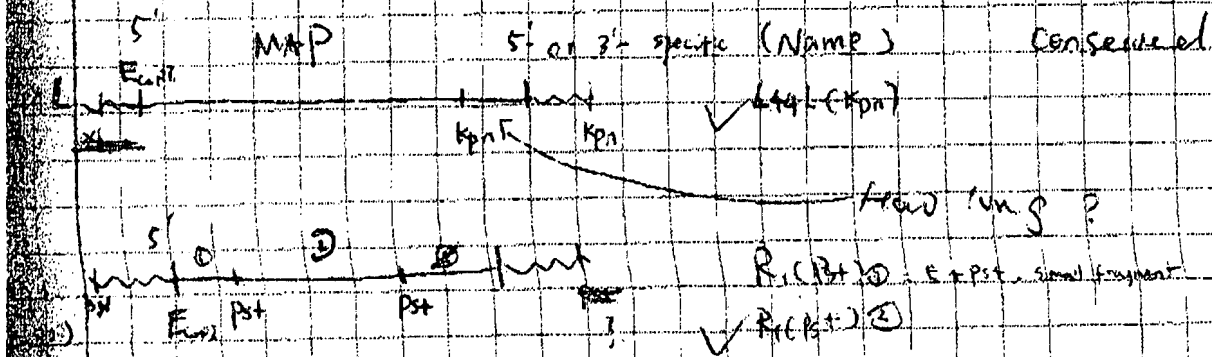
* Row gel / RNA blotting // for Log's (CC) Test

CCBT 1 - CCBT 3

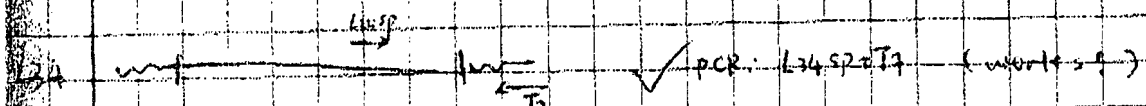
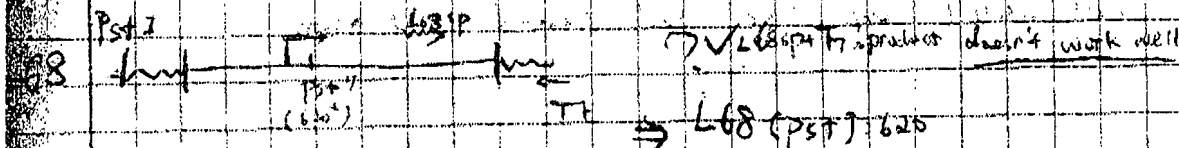
E₀ E₁ E₂ E₃ E₄ C₁ C₂ C₃ C₄ C₅ C₆ B₁ B₂ B₃ B₄ B₅ A₁ A₂ A₃ A₄ A₅ A₆ V₀ V₁ V₂ V₃ V₄

Q130
AM

Generating Gene-specific probe (for Northern) or Conserved probe / screening homologs



(note: 2012 covers domain X, XI, so it is not gene-specific)



Rekenul Trial Proposal for NOVATIS CORPORATION

Jan 10

(1) Northern blotting

ABA - BTH - JA

(3 x 7: 0, 1/2, 1, 2, 4, 6, 12, h.)

SA - Wounding - AWR - vir

7 7 5 (0, 1, 2, 4 drops)

2 sets

Blots name - All-in-One 1st, 2nd

(2) Southern blotting

3µg digested by EcoRI Hind III
(perfect digestion)

2 tubes { 150 µg/ml X 600 µl
600 µg/ml X 500 µl
New DNA from Dren using CTAB method

SEH-5 -6 -7 -8

Repeat

repeat from ligation

(3) Fusion construction → ligation → transformation

(see Jan 3 for detail)

(4) Picture attached:

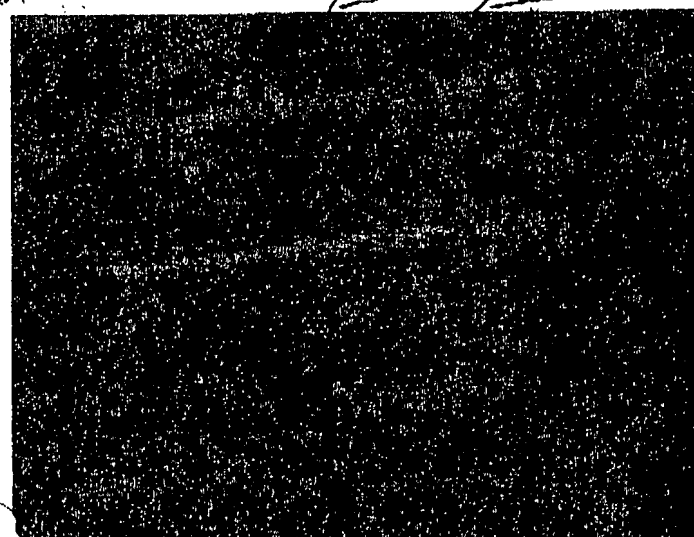


EXHIBIT B

Department Plant Pathology
 Subject Rice Defense gene Characterization
 Name Lizhang Xiong
 Address Rose APC 215

National® Brand

2001.3

Computation Notebook

11 3/4" x 9 1/4", 4 x 4 Quad., 75 Sheets

43-648



0 73333 43648 8

II



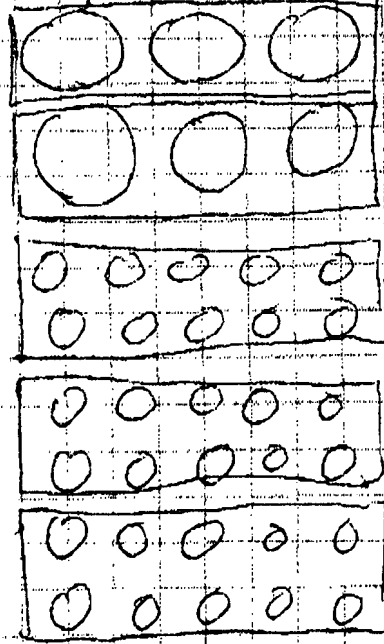
Office Products
 Chicopee, MA 01022

5.18. Summary of Transformation efficiency

Construct	Resistant calli		Shoots obtained	
		Total		Total
① F ₂ -N	18/37 12/41 :	84/276	/	
G ₂ -N	24/29 22/32 17/26 :	119/301		
⑩ H ₂ -N	26/41 28/42 :	107/244		
H ₂ -D	3/46 1/42 :	7/263		
C ₃ -D	0/52 2/47 :	9/312		
C ₃ -N (?)	2/36 0/- 4/51 :	7/282		
G ₂ -HJ	1/40 2/40 2/38 0/- 0/-	9/317		

* Planting Seeds

W ↑



← ~~Nipponbare~~ Nipponbare

← Dread

Purpose: Stress Test for M_p * large pots are control for transgenic line

- 1* Salt 150-200 mM NaCl
Root & leaf 0, 1, 3, 6, 12, 24, 72hr, 7d
- 2* Cold $28^\circ \rightarrow 4^\circ$:
0, 3h, 6h, 12h, 24h
or $28^\circ \rightarrow 24^\circ$ for 24h $\rightarrow 28^\circ$
0, 3h, 6h, 12h, 24h

- 3* Drought
Stop water supply (wet soil)
0 day, 1d, 2, 3d, 4d ← water content

- 4* Senescence (chlorophyll content?)

* sampling: small scale in 1.5ml tube (RNA)
medium scale in 15ml tube (protein)

D. TRIAL Western / plant protein
 → ABA induced. Wounding induced. blast fungus induced
 → extracted w/ Lab. protocol for tobacco.

E. TRANSGENIC "F₂" (M₂ - DsRNAi)

2 Stages Experiment

STAGE I: COPY NO. (Southern) and Expression of DsRNAi | enzyme
 screening all 40 lines
 1st Hybridized w/ sequ on DsRNA
 2nd " " w/ sequ not on DsRNA

STAGE II: Matured plants (w/ 1 copy and expressed DsRNA)

* leaf segment → blast fungus (Dot inoculation)
 (Note: not 15/1, ask min for fungus)

* Intact leaf on plant → spray ABA

other treatment using leaf segment if possible

* phenotype Recording for all lines (all constructs)

Only lines showing that ^{endogenous} M₂ is inhibited to be induced
 carry on to T₁ generation

F. TRANSGENIC "H₂-N / H₂-D₁

STAGE I: Same as "F₂"

STAGE II: Same as "F₂" (Focus on blast fungus)

Expected lines: Enhanced Resistance

G. TRANSGENIC Line "G₂" - L₂ DsRNAi

STAGE I: Same as in E except

Sampling for ABA at both 8 AM and 9 PM

endogenous species

6.7 1st Transfer seedlings (H₂ #) (3-N #)

2 Sampling: Cold - RC - 24hr.
Salt 48h. (leaf & root) | AM

Drought PMS: w (2 day)
plus F₂-1 - 22

3 Extract RNA for all samples. Conc. not determined.

4 PCR for M₂ - deletion / splicing

Drew, Drews plasmid M₂ plasmid M₂ H₂O

primer: RTM₂F RTM₂R (product length ^{from} of M₂ should be 1.0.

Taq: Anne made (asul) in 50ul vol
added after temp. reach 95°C

6.8 1 CK PCR

2 Transfer E₁₆-H₂ (only one) Resistant callus to Regeneration Medium

3 Salt 3d.
drought 3d. | Sampling

4 prepare tissue in Mon (S₂RK)